Distribution of ^{14}C-labeled metabolites in mycorrhizal and nonmycorrhizal lodgepole pine seedlings[1]

J. R. AHRENS AND C. P. P. REID
Department of Forest and Wood Sciences, Colorado State University, Fort Collins, Colorado

Received December 6, 1972

AHRENS, J. R., and C. P. P. REID. 1973. Distribution of ^{14}C-labeled metabolites in mycorrhizal and nonmycorrhizal lodgepole pine seedlings. Can. J. Bot. **51**: 1029–1035.

Four days after $^{14}CO_2$ assimilation, the distribution of ^{14}C-labeled metabolites in mycorrhizal and nonmycorrhizal pine seedlings was investigated by use of autoradiography, thin-layer chromatography, and liquid scintillation techniques. Mycorrhizal formation was accomplished by growing the lodgepole pine (*Pinus contorta* Dougl.) seedlings in axenic culture with the fungal symbionts *Thelephora terrestris* (Ehrh.) Fr., *Cenococcum graniforme* (Sow.) Ferd. and Winge, and *Rhizopogon vinicolor* A. H. Smith.

Although no significant differences between ectomycorrhizal and uninfected pines were found in the distribution of ^{14}C-labeled metabolites after seedling harvest, important trends were noted in both seedling types with respect to the ^{14}C distribution. Total radioactivity within both mycorrhizal and nonmycorrhizal seedlings was highly correlated with shoot dry weight. Most activity was present in ethanol-soluble fractions. The percentage of activity in ethanol-soluble fractions was greater in roots than shoots. Sugars formed the greatest percentage of the labeled, ethanol-soluble fractions, with sucrose the most common. The roots of all plants analyzed had higher percentages of sucrose and lower percentages of hexoses than did the shoots. Of the hexoses, glucose was more prominent in the shoots and fructose more prominent in the roots of both types of seedlings. Greater quantities of ^{14}C-labeled organic acids and amino acids were found in the shoots than in roots. Both mycorrhizal and nonmycorrhizal plants had similar mean ^{14}C activities in root exudate fractions collected in distilled water. This activity was not correlated with root dry weight or ^{14}C activity within the plant.

There was no evidence to support the hypothesis that mycorrhizal development promotes increased translocation of assimilates to the roots or results in increased soluble carbohydrate levels.

AHRENS, J. R., et C. P. P. REID. 1973. Distribution of ^{14}C-labeled metabolites in mycorrhizal and nonmycorrhizal lodgepole pine seedlings. Can. J. Bot. **51**: 1029–1035.

Par des techniques d'autoradiographie, de chromatographie en couche mince et de scintillation liquide, les auteurs ont étudié la distribution des métabolites marqués au ^{14}C, 4 jours après l'assimilation de $^{14}CO_2$, dans des plantules de *Pinus contorta* Dougl. mycorrhizées et non mycorrhizées. La production de mycorrhizes a été réalisée par la culture axénique de plantules avec les mycobiontes *Thelephora terrestris* (Ehrh.) Fr., *Cenococcum graniforme* (Sow.) Ferd. et Winge et *Rhizopogon vinicolor* A. H. Smith.

Bien qu'aucune différence significative n'ait été perçue entre les pins ectomycorrhizés et les pins non infectés, dans la distribution des métabolites marqués au ^{14}C après la récolte des plantules, des tendances importantes ont été remarquées dans les deux types de plantules relativement à la distribution du ^{14}C. La radioactivité totale chez les plantules mycorrhizées aussi bien que chez les plantules non mycorrhizées était en étroite corrélation avec le poids sec de la tige. La majeure partie de l'activité se situait dans les fractions solubles dans l'éthanol. Le pourcentage d'activité dans les fractions solubles à l'éthanol était plus élevé dans les racines que dans les tiges. Les sucres formaient le plus fort pourcentage des fractions marquées solubles à l'éthanol, le sucrose étant le plus commun. Les racines de toutes les plantes analysées avaient de plus forts pourcentages de sucrose et de plus faibles pourcentages d'hexoses que les tiges. Parmi les hexoses, le glucose était plus abondant dans les tiges et le fructose plus abondant dans les racines des deux types de plantules. De plus fortes quantités d'acides organiques et d'acides aminés marqués au ^{14}C ont été retrouvées dans les tiges que dans les racines. Les plants mycorrhizés et les plants non mycorrhizés avaient des niveaux moyens de radioactivité semblables dans les fractions d'exsudats racinaires récoltées dans l'eau distillée. Cette radioactivité n'était pas en corrélation avec le poids sec des racines ni avec la radioactivité due au ^{14}C à l'intérieur de la plante.

Aucune preuve n'a été trouvée pour supporter l'hypothèse que le développement des mycorrhizes augmente le translocation des produits de la photosynthèse vers les racines ou entraîne une augmentation des niveaux de glucides solubles. [Traduit par le journal]

Introduction

Current evidence indicates that the invasion of roots by mycorrhizal fungi leads to metabolic changes within the root. Studies by Nelson (21) indicated that red pine (*Pinus resinosa* Ait.) translocated significantly more assimilates to mycorrhizal roots than to nonmycorrhizal roots. Meyer (19) cited studies that also suggested that the amount of soluble sugars in mycorrhizal roots increased as the degree of mycorrhizal infection increased.

[1]Supported by National Science Foundation Grant GB-19928.

Lewis and Harley (14, 15, 16) investigated the influence of fungal infection on the compounds present in the excised ectomycorrhizal roots of European beech (*Fagus sylvatica* L.). They found such compounds as trehalose, mannitol, and glycogen to be characteristic of the mycorrhizal fungus but not the host tissue. Ectomycorrhizal roots of many tree species are unlike those of beech, in that the fungal mantles of other species are less thick and more difficult to separate from the host root. Any analysis of assimilates present in such mycorrhizal roots, by necessity, has to be done without the separating of root and fungal tissue.

The ecological role of mycorrhizas in carbohydrate movement beneath the forest floor is not completely known. Work involving carbohydrate transport via mutually shared mycorrhizas (1, 25) suggests that individual trees joined by mycorrhizas and fungal mycelia may not function independently of one another as discrete autecological units.

This study investigated possible quantitative and qualitative differences between ectomycorrhizal and nonectomycorrhizal pine seedlings in their assimilation and distribution of ^{14}C-labeled metabolites. This is a necessary first step in determining the role of forest tree mycorrhizas in providing a mechanism for the transport or exchange of root metabolites between trees of a forest stand.

Methods and Materials

Mycorrhiza Synthesis

Mycorrhizas were synthesized by axenic culture techniques. This involved growing the seedlings aseptically within a previously sterilized container/substrate system (2, 10, 18). Six hundred milliliters of the coarse fraction of screened vermiculite thoroughly mixed with 40 ml of the fine fraction of screened peat moss was added to half-gallon jars (10, 18). Two different nutrient solutions were used. To the substrates was added four hundred milliliters of either a modified Melin-Norkrans nutrient solution, based on the formulation presented by Marx (17): 0.05 g $CaCl_2$, 0.025 g NaCl, 0.5 g KH_2PO_4, 0.25 g $(NH_4)_2HPO_4$, 0.15 g $MgSO_4 \cdot 7H_2O$, 0.005 g Sequestrene 330 Fe (14.2% Fe as Fe_2O_3), 10.0 μg thiamine-HCl, 10.0 g glucose, 5.0 g malt extract, and distilled H_2O to 1000 ml; or a modified nutrient solution originally developed by N. M. Shemakhanova and used by Trappe (30): 0.05 g $CaCl_2$, 0.25 g K_2HPO_4, 0.15 g $MgSO_4 \cdot 7H_2O$, 0.005 g Sequestrene 330 Fe, 50.0 μg thiamine-HCl, 5.0 g glucose, and distilled H_2O to 1000 ml. The jars were capped with cotton plugs and a layer of aluminum foil, then sterilized in an autoclave for 30 min at 121°C.

Lodgepole pine (*Pinus contorta* Dougl.) seeds of Colorado origin were surface-sterilized, germinated on cornmeal agar, and planted aseptically in the previously described substrate.

About one-half of the containers were inoculated with mycelial disks of ectomycorrhizal fungi that had been maintained on agar media. Although isolates of eight fungal species were used for these inoculations, three—*Cenococcum graniforme* (Sow.) Ferd. and Winge, *Rhizopogon vinicolor* A. H. Smith, and *Thelephora terrestris* (Ehrh.) Fr.—gave the best mycorrhiza formation and were used in forming of mycorrhizas of the seedlings used in subsequent ^{14}C studies.

Any containers showing signs of contamination either before or after inoculation were discarded. Both the inoculated and uninoculated cultures were kept in an insulated greenhouse waterbath where the temperature was kept within a range of 18–25°C. Fifty-five percent shade cloth reduced radiation intensity and kept container temperatures lower during midday.

Introduction of $^{14}CO_2$

Seedlings were selected for ^{14}C studies when about 5 months old. The selected seedlings were gently cleansed of any clinging root debris by rinsing them in water and using an ultrasonic cleaner (4). The plants were then placed in 400 ml of aerated, distilled water, and allowed to equilibrate in a growth chamber for 24 h before $^{14}CO_2$ exposure. The chamber was set for a 6-h photoperiod, with light supplied by both cool white fluorescent and incandescent bulbs which yielded a far-red to blue spectral energy of 1070 μW cm^{-2}. The day–night temperature regime was 23 and 16°C, respectively.

At the begining of the light period, the seedling foliage was enclosed within an assimilation chamber such that the shoot was isolated from the roots. One hour later, 10 μcuries of ^{14}C was applied to the plant as $^{14}CO_2$ by acidifying a $NaH^{14}CO_3$ solution. The assimilation chamber was removed 2 h later.

Seedling Harvest and Separation

Time-course data dictated termination of the assimilation period 96 h after the introduction of $^{14}CO_2$. This allowed significant radioactivity to be distributed throughout the root system.

After the assimilation period, the root-bathing distilled water (exudate fraction) was evaporated under vacuum at 40°C to 10 ml and sampled for total radioactivity.

The plant was divided into a shoot and root portion after a distilled H_2O rinsing and blotting between absorbent towels. The degree of mycorrhizal formation of the inoculated seedlings was determined by obtaining the percentage of short roots exhibiting dichotomy (11, 29). Samples of the dichotomous short roots were preserved in the formalin, glacial acetic acid, 70% ethanol (1:1:18 v/v) solution of Johansen (12) for histological examination and confirmation of the mycorrhizal sheath and Hartig net (7, 25). Fresh weights were obtained for each portion. These fresh weights were later converted to dry weights for use in specific activity determinations, by using fresh weight – dry weight ratios obtained in earlier work.

Extraction and Fractionation of Ethanol-soluble Compounds

The roots and shoot (hereafter meant to include both the needles and stem) were next dropped into hot 80% ethanol for 3 min, macerated in a Waring blendor for 7 min, and then successively extracted with 80%, 50%, and 80% ethanol (3, 28). The shoot extract was cleared of chlorophyll, which otherwise acted as a quench in subsequent liquid scintillation countings, by swirling the extract with activated carbon for 10 min. Appropriate procedures and calculations were conducted to determine any loss of radioactivity.

The remaining ethanol-insoluble residues were assayed for radioactivity after digesting them with 60% $HClO_4$ and 30% H_2O_2 (25).

Dowex 50W-X4 resin (50–100 mesh, H^+ form) and Dowex 1-X8 resin (50–100 mesh, formate form) were used to separate each ethanol-soluble extract into organic acid, amino acid, and sugar fractions (23, 28). Each of these fractions was then sampled for total radioactivity.

TABLE 1

Distribution of ^{14}C-labeled compounds in the shoots and roots of mycorrhizal and nonmycorrhizal lodgepole pine seedlings

	Means ± SE	
	Mycorrhizal	Nonmycorrhizal
% mycorrhizal infection[a]	11.20±4.57	0.0
Shoot oven-dry wt., g	0.153±0.028	0.151±0.049
Root oven-dry wt., g	0.120±0.021	0.111±0.020
Root-to-shoot ratio (based on oven-dry wt.)	0.87±0.14	0.87±0.25
Fraction-specific activities ($\times 10^{-3}$) (cpm g^{-1} oven-dry wt. of extracted tissue)		
Shoot		
Insoluble	848.3±182.9	1442.9±327.1
Soluble		
Sugars	1593.1±358.3	1551.0±239.7
Organic acids	500.6±119.8	706.3±141.3
Amino acids	88.2±15.0	86.9±25.1
Root		
Insoluble	129.0±31.1	232.2±36.6
Soluble		
Sugars	637.5±159.3	981.4±119.9
Organic acids	92.6±28.1	141.4±17.4
Amino acids	76.6±21.5	66.6±15.3
% of total ^{14}C-activity in soluble fraction[b]		
Shoot	70.9±3.7	67.7±4.5
Root	85.8±1.5	83.5±2.3
% of total ^{14}C-activity in root	20.62±3.59	30.81±7.53
Total ^{14}C-activity in root exudates, cpm[c]	1007±513	1141±386

[a]Based on percentage of short roots exhibiting dichotomy.
[b]Percentage in soluble fraction calculated from total of ethanol-soluble and ethanol-insoluble specific activities in each individual plant part.
[c]Exudates collected in root-bathing media of each plant.

The sugar fraction was further broken down by using thin-layer chromatographic techniques. A solvent system of formic acid, methylethylketone, isopropanol, and water (15:30:40:15 v/v) was used in conjunction with cellulose chromatographic sheets. Each chromatogram was run twice in an ascending direction.

Unknown sugars were located through the use of autoradiographic methods (6). The located unknowns were scraped from the chromatograms into scintillation vials and the radioactivity determined.

Liquid Scintillation Determination of Radioactivity

A liquid scintillation spectrometer (Packard Instrument Co., Inc., Model 2420, Tri-carb) was used in all determinations of ^{14}C activity. Quench curves were prepared by plotting an automatic external standardization ratio against the counting efficiency percentage obtained from tests with known amounts of ^{14}C-toluene.

The scintillator solvent systems used depended on the nature of the substance containing the ^{14}C. An Insta-gel (Packard Instrument Co., Inc.) scintillator solvent was used with aqueous samples. Ten milliliters of the Insta-gel was added to 1 ml of the aqueous samples to be counted. The insoluble residues that were dissolved in 60% $HClO_4$ and 30% H_2O_2 were mixed with 15 ml of a toluene–cellosolve solution prepared according to Reid and Woods (25). Thin-layer chromatogram scrapings were suspended in 15 ml of a thixotropic gel (Cab-O-Sil: Cabot Corp.) scintillator solution prepared according to a formulation outlined in the 1971 Packard Catalog.

To determine whether or not a significant difference existed between the radioactivity of an unknown sample and natural background, a *t*-test of significance was used (5):

$$t = (r_s - r_b) \,/\, (r_s/t_s + r_b/t_b)^{1/2}$$

where t is the relative error, r_s is the count rate of the sample, r_b is the count rate of the background, t_s is the length of time the sample was counted, and t_b is the length of time the background was counted.

In the analysis of ^{14}C-related data, the seedlings were placed in either a mycorrhizal or nonmycorrhizal category. Although seedlings in the mycorrhizal category were associated with any one of three fungal symbionts and had varying degrees of fungal infection, no attempt was made to distinguish these characters during data analysis, except where noted.

Results

Distribution of ^{14}C within Seedlings

A summary of experimental results obtained from six mycorrhizal and seven nonmycorrhizal lodgepole pine seedlings is presented in Table 1. Although not shown in Table 1, the mean plant net radioactivity (cpm) recovered within the nonmycorrhizal group was 18% higher than that recovered within the mycorrhizal group. This percentage was not statistically significant, but it undoubtedly contributed to the somewhat higher specific activities for the soluble and

insoluble fractions of the nonmycorrhizal plants since root and shoot weights and ratios were similar in the two groups. The net counts per minute within any particular plant was closely correlated with the dry weight of the plant shoot, as shown by a Pearson's test ($r = +0.94$). The percentage of net counts per minute within the roots showed a good correlation ($r = +0.89$) with the root-to-shoot ratio but no significant correlation with the percentage of mycorrhizal infection.

The specific activities of the fractions, shown in Table 1, are based on the counts per minute per gram oven-dry tissue extracted. The percentage of activity found in the soluble fraction of both shoots and roots of mycorrhizal plants was slightly higher than that of nonmycorrhizal plant shoots and roots, but the difference was not significant at the 5% level. If the values from all plants (both mycorrhizal and nonmycorrhizal) are considered together, the proportion of ^{14}C in the ethanol-soluble fraction is about 15 percentage points greater in roots than shoots. It was calculated that only a small percentage of this difference could be attributed to the loss of counts during the clearing of shoot extracts with activated carbon.

The soluble fraction is presented as the mean specific activity of each class of compounds: sugars, organic acids, or amino acids. Since it was apparent that the mean uptake of $^{14}CO_2$ by the mycorrhizal plant group was less than that of the nonmycorrhizal plant group, statistical comparisons were made after the mean specific activity of each compound class was expressed as a percentage of the total soluble specific activity found in each plant. No significant (5% level) differences were found between mycorrhizal and nonmycorrhizal roots or shoots when the mean percentages of the ^{14}C-labeled classes of compounds were compared. For both groups of seedlings, the sugar fraction greatly predominated in both shoots and roots, followed in descending order by organic acids and amino acids.

Although the means of ^{14}C present in shoot and root insoluble fractions of mycorrhizal and nonmycorrhizal pines are shown in Table 1, it is interesting to note that if the specific activities of the individual mycorrhizal plants are plotted, both the ethanol-soluble and ethanol-insoluble fractions generally decrease curvilinearly with the increasing infection percentages (Figs. 1 and 2). This relationship appears somewhat more consistent for the root values than shoot values.

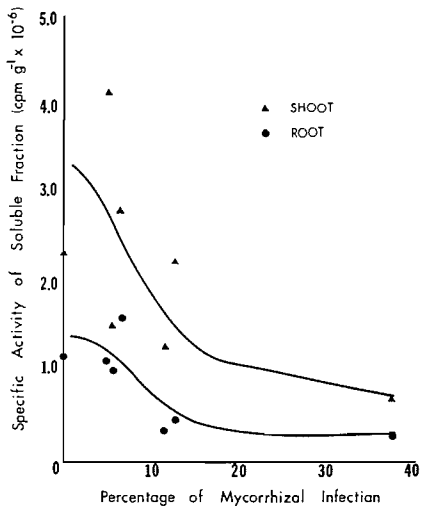

FIG. 1. Relation between specific activity of root and shoot ethanol-solubles and percentage of mycorrhizal infection in mycorrhizal lodgepole pine seedlings harvested 4 days after initial exposure to $^{14}CO_2$. Values at 0% represent average of seven nonmycorrhizal plants.

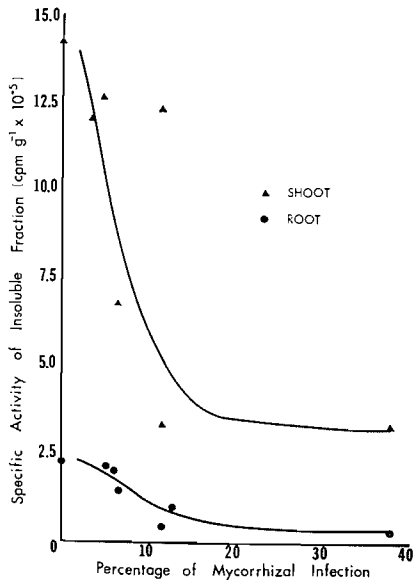

FIG. 2. Relation between specific activity of root and shoot ethanol-insolubles and percentage of mycorrhizal infection in mycorrhizal lodgepole pine seedlings harvested 4 days after initial exposure to $^{14}CO_2$. Values at 0% represent average of seven nonmycorrhizal plants.

TABLE 2

Summary of percentage distribution of ^{14}C activity in individual sugars of mycorrhizal and nonmycorrhizal pines

Plant type and section	Percentage of radioactivity in:				
	raffinose	sucrose	glucose	fructose	unknown
Nonmycorrhizal					
Shoot	1.0±0.6[a]	54.1±9.2	26.2±5.0	17.7±3.1	1.0±0.9
Root	0.7±0.7	78.4±4.3	8.6±2.3	12.3±3.2	0.0
Mycorrhizal					
Shoot	0.3±0.2	62.2±4.7	18.9±2.2	16.2±1.3	2.4±2.2
Root	0.0	77.3±5.3	10.2±3.1	12.5±2.8	0.0

[a]Mean ± SE.

Distribution of ^{14}C among Compound Fractions

The relation of the labeled-compound fractions to each other was also expressed by the mean ratios of organic acids and amino acids to sugars, based on the specific activities of each. As a result of the large variation, no significant (5% level) differences were found between the ratios for mycorrhizal and nonmycorrhizal seedlings. However, when the ratios were calculated for the mycorrhizal and nonmycorrhizal plants combined as one group, the ratio of organic acid to sugar in the shoots (0.424 ± 0.073 SE) was significantly greater than that of the roots (0.146 ± 0.012 SE) at the 1% level. Conversely, the ratio of amino acid to sugar was greater in the roots (0.103 ± 0.018 SE) than in the shoots (0.060 ± 0.019 SE) but not significantly (10% level).

Distribution of ^{14}C within the Sugar Fraction

A summary of the percentage distribution of individual ^{14}C-labeled sugars is given in Table 2. No significant (10% level) differences were found between mycorrhizal and nonmycorrhizal plants when the mean percentages of each sugar were compared.

The predominant labeled sugar in the shoots and roots of both types of plants was sucrose. There was a higher percentage of sucrose in the roots and a correspondingly lower percentage of the hexose sugars, glucose and fructose. When only the mycorrhizal plants were considered, it was noted that the percentage of sucrose in the roots appeared correlated ($r = +0.91$) with the degree of mycorrhizal infection; the percentage of hexoses, of course, showed a similar correlation but with an opposite sign. Because of the limited number of samples involved, the high correlation coefficients may be only fortuitous and not indicative of a significant physiological response to infection.

No trehalose, mannitol, or inositols were detected in compounds that became labeled.

The labeled hexoses present in both types of seedlings were more prominent in the shoot. Glucose-to-fructose ratios indicated that glucose was more common in shoots but the opposite was true in roots.

^{14}C Activity in Root Exudates

Both mycorrhizal and nonmycorrhizal plant roots exuded about the same mean of ^{14}C activity (Table 1), although the variability between plants was high. This exudation activity averaged about 0.3% of the total soluble counts per minute within the plants. There was no significant correlation, even at the 10% level, between the total exudation activity and the percentage of infection, root-to-shoot ratio, root dry weight, root soluble counts per minute, or total soluble counts per minute within the plant.

Discussion

No significant differences in the distribution of ^{14}C were noted between ectomycorrhizal and non-ectomycorrhizal lodgepole pines. The mannitol, trehalose, and inositols suggested by Lewis and Harley (14) to be concurrent with the fungal infection were not found in the ^{14}C-labeled ethanol-soluble fraction. This does not preclude their presence. They may have remained unlabeled as a result of a slow turnover rate. It should also be mentioned that the work of Lewis and Harley involved ectomycorrhizas that had fungal mantles much larger than the mantles of ectomycorrhizas used in this research. Larger

mantles would cause compounds characteristic of the fungus to be present in greater proportions.

Although the ^{14}C activity, expressed either as total net counts per minute or as specific activity (cpm/g oven-dry weight), was not significantly different (at least at the 10% level) between the nonmycorrhizal and mycorrhizal groups, the means were lower in the mycorrhizal group. This may be indicative that there was less $^{14}CO_2$ fixation in the latter group.

Most of the ^{14}C activity present in all plants was found in the ethanol-soluble fraction, with the bulk of this activity located in the shoot. The concentration of ^{14}C in the shoot may attest to the ability of the young needles and terminal buds to act as sinks for the ^{14}C (9, 26). The lower percentage of activity in the ethanol-soluble fraction of shoots as compared to roots may also indicate that the shoots were using more ethanol-soluble ^{14}C in the synthesis of new tissue than the roots.

The higher specific activities of organic acids in the shoot as compared to those in the root were also most likely the result of a large amount of biosynthesis occurring in the shoot (20, 24). The predominance of amino acid activity in the roots might be explained by the fact that roots are the main center of nitrogen incorporation into translocatory amino acids (13). The amino acids translocated to the shoot were probably converted quickly to proteins (27).

The lower glucose-to-fructose (less than 1.00) ratio in the roots is in line with the findings of others (15, 22, 24). The glucose-to-fructose ratio greater than 1.00 found in the shoots varies somewhat from other findings (8, 24). No concrete conclusions can be drawn regarding this hexose distribution, only speculation, especially in the absence of information on pool size or relative turnover rates of individual compounds. It appears that a different enzyme system exists in the shoots than in the roots. Sluggish or inhibited isomerases and hexokinases may well have been involved; compartmentalization could also cause the selective increase of one hexose over the other.

These findings do not support the hypotheses (19, 21) that mycorrhizal development promotes increased translocation of assimilates to the roots or results in increased soluble carbohydrate levels.

1. BJÖRKMAN, E. 1949. The ecological significance of the ectotrophic mycorrhizal association in forest trees. Sven. Bot. Tidskr. **43**: 223–262.
2. BRYAN, W. C., and B. ZAK. 1961. Synthetic culture of mycorrhizae of southern pines. For. Sci. **7**: 123–129.
3. CANVIN, D. T., and H. BEEVERS. 1961. Sucrose synthesis from acetate in the germinating castor bean: kinetics and pathway. J. Biol. Chem. **236**: 988–995.
4. CARLSON, L. W., and J. BELCHER. 1970. Effect of ultrasonic root cleaning on subsequent growth of Caragana seedlings. Can. For. Serv. Bimon. Res. Notes, **26**: 27.
5. CHASE, G. D., and J. L. RABINOWITZ. 1967. Principles of radioisotope methodology. Burgess Publ. Co., Minneapolis.
6. CRAFTS, A. S., and S. YAMAGUCHI. 1964. The autoradiography of plant materials. Univ. Calif., Davis.
7. FORTIN, J. 1966. Synthesis of mycorrhizae on explants of the root hypocotyl of *Pinus sylvestris* L. Can. J. Bot. **44**: 1087–1092.
8. GEIGER, D. R., and J. W. BATEY. 1967. Translocation of ^{14}C sucrose in sugar beet during darkness. Plant Physiol. **42**: 1743–1749.
9. GORDON, J. C., and P. R. LARSON. 1968. Seasonal course of photosynthesis, respiration, and distribution of ^{14}C in young *Pinus resinosa* trees as related to wood formation. Plant Physiol. **43**: 1617–1624.
10. HACSKAYLO, E. 1953. Pure culture syntheses of pine mycorrhizae in terra-lite. Mycologia, **45**: 971–975.
11. HARLEY, J. L. 1969. The biology of mycorrhiza. Leonard Hill, London.
12. JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill Book, Co., Inc., New York.
13. KRAMER, P. J., and T. T. KOZLOWSKI. 1960. Physiology of trees. McGraw-Hill Book Co., Inc., New York.
14. LEWIS, D. H., and J. L. HARLEY. 1965. Carbohydrate physiology of mycorrhizal roots of beech. I. Identity of endogenous sugars and utilization of exogenous sugars. New Phytol. **64**: 224–237.
15. LEWIS, D. H., and J. L. HARLEY. 1965. Carbohydrate physiology of mycorrhizal roots of beech. II. Utilization of exogenous sugars by uninfected and mycorrhizal roots. New Phytol. **64**: 238–255.
16. LEWIS, D. H., and J. L. HARLEY. 1965. Carbohydrate physiology of mycorrhizal roots of beech. III. Movement of sugars between host and fungus. New Phytol. **64**: 256–269.
17. MARX, D. H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology, **59**: 153–163.
18. MARX, D. H., and B. ZAK. 1965. Effect of pH on mycorrhizal formation of slash pine in aseptic culture. For. Sci. **11**: 66–75.
19. MEYER, F. H. 1966. Mycorrhiza and other plant symbioses. *In* Symbiosis. Vol. I. *Edited by* S. M. Henry. Academic Press, New York. pp. 171–255.
20. MEYER, M. M., JR., and W. E. SPLITTSTOESSER. 1969. The utilization of carbohydrate and nitrogen reserves in the spring growth of lilac. Physiol. Plant. **22**: 870–879.
21. NELSON, C. D. 1964. The production and translocation of photosynthate-C^{14} in conifers. *In* The formation of wood in forest trees. *Edited by* M. H. Zimmermann. Academic Press, New York. pp. 243–257.
22. PEEL, A. J. 1970. Movement of sugars and phosphates into sieve elements of willow. *In* Physiology of tree crops. *Edited by* L. C. Luckwill and C. V. Cutting. Academic Press, New York. pp. 129–141.

23. PLAISTED, P. H. 1958. Clearing free amino acid solutions of plant extracts for paper chromatography. Contr. Boyce Thompson Inst. Plant Res. **19**: 231–244.
24. RANGNEKAR, P. V., and D. F. FORWARD. 1969. Foliar nutrition and growth in red pine: the fate of photoassimilated carbon in a seedling tree. Can. J. Bot. **47**: 897–906.
25. REID, C. P. P., and F. W. WOODS. 1969. Translocation of C^{14}-labeled compounds in mycorrhizae and its implications in interplant nutrient cycling. Ecology, **50**: 179–187.
26. SCHIER, G. A. 1970. Seasonal pathways of ^{14}C-photosynthate in red pine labeled in May, July, and October. For. Sci. **16**: 2–13.
27. SPENCER, P., and J. S. TITUS. 1971. Translocation of glutamate-^{14}C and aspartate-^{14}C by intact apple trees. J. Am. Soc. Hortic. Sci. **96**: 131–133.
28. SPLITTSTOESSER, W. E. 1969. Arginine metabolism by pumpkin seedlings. Separation of plant extracts by ion exchange resins. Plant Cell Physiol. **10**: 87–94.
29. THEODOROU, C., and G. D. BOWEN. 1971. Influence of temperature on the mycorrhizal associations of *Pinus radiata* D. Don. Aust. J. Bot. **19**: 13–20.
30. TRAPPE, J. M. 1967. Pure culture synthesis of Douglas-fir mycorrhizae with species of *Hebeloma*, *Suillus*, *Rhizopogon*, and *Astraeus*. For. Sci. **13**: 121–130.